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Chapter 46

Laboratory methods for the identification of soft corals (Octocorallia: Alcyonacea)

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ABSTRACT
Fleshy soft corals have become a popular component of modern live coral displays. Identification of wild specimens is often based on photograph references. Many of these octocorals will undergo some form of morphological variation with time under captive conditions. Identifications beyond the family level based on the appearance of gross forms become difficult at best, if not impossible. Examining skeletal components with a light microscope is the technique used to perform family, genus, and species level classification. This paper demonstrates the taxonomic identification process of light microscopy. It provides an introduction to the fundamental differences among genera, illustrates modern techniques, and identifies many of the difficulties facing the current status of octocoral taxonomy. Offered for consideration are suggestions for contributions by public aquaria to improve our collective knowledge of octocoral biogeography.

INTRODUCTION

A key component to investigating cnidarians, or more specifically soft coral systematics is the opportunity to examine the composition of specimens. Studies made of living specimens in the wild provide little information beyond some gross morphological features that can be recorded from a brief encounter. Aquaria provide a longer period for investigation because the animals can be observed or photographed on an almost continuous basis; however, the living coral in situ offers only its gross morphology and sometimes a hint of internal details when tissues are semi-transparent. The most productive form of identifying octocorals is to examine preserved specimens.

Since the early beginnings of octocoral systematics, dating back over one hundred years ago (Bayer, 1995 www1), it has been necessary to collect and preserve specimens. Not only was preservation needed to keep specimens from rotting during their long journey to far off museums and universities, but also to allow many different investigators the opportunity to study them in the years that followed. Today, museums and research institutions maintain vast collections of preserved specimens that continue to be studied.

Light microscopy remains the primary means for identifying octocorals to the genus and species levels. Basic light microscope techniques are invaluable and can be used to perform accurate identification of octocoral families and genera. Many Indo-Pacific Alcyonacean genera have distinct sclerite forms, arrangements, or distributions within the colony tissue that make their identification possible. Although this paper focuses mainly on the family Alcyoniidae with examples, sclerite forms and discussion the techniques presented here are applicable to other octocoral families, including gorgonians. In recent years, scanning electron microscopy (SEM) has been used to investigate in greater depth and detail the surface and internal structure of sclerites. The results of SEM work and other tools such as histology, have lead to changes in some classical division in octocoral systematics (Grasshoff, 2000; Alderslade, 2001).

Today, we have the advantage of digital photography that makes it possible to quickly record images of living species. These photographs combined with habitat data, gross morphology and microscopic surveys provide a much more detailed taxonomic description than available in much of the published octocoral descriptions of the twentieth century (Kukenthal, 1923; Roxas, 1933a, 1933b). Public aquarium

personnel are well positioned with the tools, specimens, and opportunities for collection of wild specimens to contribute to our knowledge base of octocoral taxonomy. The advent of Internet resources and electronic communication also affords convenient access to professionals and institutions where taxonomists can assist them with the identification process. The methods and general overview provided here allows aquarists to confidently collect microscopic data on octocoral genera.

MATERIALS

The process of examining octocoral specimens for identification requires some fundamental laboratory equipment. Additionally, various chemicals are required to anesthetize and fix specimens, and isolate sclerites, all of which are available from chemical supply sources. As with any work involving chemicals and specialized equipment, care should be given to proper safety procedures and expert guidance. It is also important to review proper safety precautions, seek out the advise of a qualified laboratory technician, and handle or dispose of chemicals in accordance with local laws and regulations.

A basic dissection kit will contain most of the tools necessary for sampling octocoral sclerites. Teasing needles or fine-pointed dental picks are helpful when working with sclerites on glass microscope slides. Also useful are fine tipped forceps, like those used by jewelers work well to manipulate individual sclerites for light microscopy or when preparing SEM stubs. Scalpels with disposable blades are an economic choice for sampling soft corals. Blades often wear quickly, becoming dull when used to cut tissue containing sclerites, gorgonin, or calcareous material. Keep a few extra replacement blades on hand for when the need to replace arises.

Both compound and stereomicroscopes are regularly used to investigate octocorals. Stereomicroscopes with moderate magnification up to twenty-five power and good lighting make it possible to examine the finer details of colony morphology, calyces, polyp armatures, and internal structures such as the axis formation in gorgonians. A compound microscope is necessary to observe sclerite surface details and to measure the size of sclerites. Typically, ten power oculars in conjunction with a ten-power objective provide appropriate magnification for most sclerites. Occasionally, a lower four-power objective may be used to place the largest sclerites into the field of view. A higher magnification of four hundred, a ten-power ocular combined with a forty-power objective, is used to observe smaller sclerites or fine surface details.

Among the various chemicals used to prepare soft corals for identification, Ethyl alcohol requires the largest quantity. Large octocoral specimens may require a liter or more of the ethanol for preservation. There are many grades of ethyl alcohol and the more diluted the alcohol is the less effective it is at long term preservation. Any ethanol less than fifty percent can result in degraded specimens over time. Seventy percent works well for storage and ninety percent or higher is ideal for molecular studies. There is a trade off though with the higher grades of ethanol being more expensive, which can be cost prohibitive when working within a tight budget.

METHODS

Preparation of octocorals either from the wild or those taken from the aquarium for examination requires anaesthetization, fixation, preservation, sclerite isolation, and possibly permanently mounted microscope slides. With care and attention, each of these steps can be done in a relatively short period of time. Resulting in preserved specimens that can be examined at a later date, shared with other institutions or researchers, and serve as a permanent record of a soft coral that over time, may no longer populate a display or region. The following methods have been used with octocorals for many years. Although not exhaustive, these procedures offer a concise sequence that will result in specimens ready for taxonomic investigation.

Anaesthetization

In octocorals, just handling or any disturbance of a colony is enough to cause the contraction or complete retractions of polyps into the coenenchyme. To overcome this obstacle, a means of anaesthetization may be needed to retain the polyps in their natural state of expansion. Certainly contracted polyps can be differentiated in sections examined microscopically, but there are situations where a fully expanded polyp will need to be investigated. There are many different

methods for narcotizing or anaesthetizing marine invertebrates. Experiments in the early nineteen hundreds with anaesthetizing agents included the use of menthol, chloroform, alcohol, formalin, magnesium sulfate, and cocaine (Gohar, 1937). Through experimentation, many were found at that time to be unsuitable either because of potential damage to the specimen or in the case of the latter, its obvious unlawful use. Today, magnesium sulfate is the preferred choice in chemical narcotizing or relaxing octocoral polyps.

This procedure is very effective in retaining polyp expansion, even in species of *Briareum*, which often retracts the polyps when disturbed. Whenever this process is used it is important that the specimen or specimens being anaesthetized are allowed to relax and extend their polyps prior to chemical additions. Typically, a volume of seawater ten to thirty times that of the specimen is needed. Also, placing a lamp over the container holding the colonies can draw out polyps in photosynthetic corals. Exercise caution to be sure the lamp is placed high enough above the vessel to keep excessive heat from being introduced into the water. Keeping the water still without circulation or surface agitation will reduce the available oxygen that will often draw polyps further out. Sometimes a few drops of plankton foods (i.e. phytoplankton or marine snow) or liquid juices from frozen foods can be added to encourage polyp extension.
It is also possible to keep contractile specimens in a relaxed state with the use of formalin. A very diluted solution is added to a large volume of seawater containing the specimen.

First, the specimen is allowed to relax in its container. A light can be placed over the specimen to draw the polyps out, as in the previous method. Then the narcotizing solution is added to the container as far as possible from the coral so that there is no direct contact. Through slow additions over a period of time, the specimen is relaxed with little or no visible contraction.

Fixation

Once coral samples have been removed from their aquatic environment, a procedure is needed to retain the integrity of the tissue. Upon removal, naturally occurring enzymes begin to breakdown cells in a process known as autolysis. A fixative solution is used to denature or cross-link the proteins. This preserves the structure and composition of soft tissues. If an anaesthetizing agent has been used, then specimens should be removed from these chemicals and placed into a plastic or glass

Magnesium sulfate solution

5 g	Magnesium sulfate
45 ml	De-ionized water

Combine in a glass flask and mix until completely dissolved. Allow to rest until the solution reaches room temperature. Add the solution in 5 ml increments at 15-20 minute intervals to the specimen maintained in seawater. Continue additions until the specimen is unresponsive to touch. Remove from the anaesthetizing agent and place into fixative.

Formalin solution

2 ml	37% Formalin
100 ml	De-ionized water

Combine in a glass flask and mix gently. Place the specimen in a volume of seawater 50-100 times that of its own volume. Add 2-3 drops of the solution with a pipette every fifteen minutes. Additions may be doubled at one-hour intervals. The procedure is continued until the specimen is completely unresponsive. This may take several hours.

vessel for fixation.
The best method for sclerite examination is to place specimens directly in seventy to ninety-five percent ethanol. It will preserve both tissue and sclerites indefinitely. However, soft corals often shrink considerably upon contact with the ethanol, resulting in contorted specimens that can be difficult to work with. A popular method that works well for general investigation and light microscopy is formalin fixation. This method is widely used in fieldwork where there is little time or space for lengthy chemical procedures (Beneyahu, 1993; Reinicke, 1997). The down side to using Formalin is that some of the fine nuclear detail can be lost for histological work, it is unsuitable for DNA examination, and due to the acidic nature of formaldehyde it will erode sclerite details over time if any residual formalin exists (Fabricius and Alderslade, 2001).

Another method for preserving soft corals is the use of Helly's Modified Seawater fixative. This fixing solution yields very good nuclear detail and does not interfere with stains used in histological sectioning (Peters, 2000). The term "orange death" is very applicable to this solution because of its dark orange color. With proper attention to fixing times and rinsing, this fixative produces excellent results without any residual color remaining in the specimen. If the specimen has not been rinsed enough then the ethanol the specimen is stored in will take on a light orange color and should be changed out for new ethanol. The fixative has a long shelf life when stored in an air tight, glass jar. However, once the formalin has been added it only remains active for about twenty-four hours.

Preservation
Fixed samples should be stored in vials or glass jars large enough to allow the specimen to fit without contorting its shape too much. For larger specimens and some gorgonians the sample can sometimes be gently folded to fit. The storage containers should hold enough volume that specimens can be completely submerged in ethanol. A ninety-five percent ethanol is ideal however seventy percent ethanol is sufficient

Formalin fixation

20 ml 37 % Formalin
500 ml Filtered seawater or Synthetic seawater

Place the specimen in a glass vessel containing the seawater. Gently add the formalin to the container and stir gently. Try to disturb the coral as little as possible. Cover the container and leave for 24 hours. Remove the specimen from the formalin solution and rinse in running fresh water for 5-12 hours. Preserve in ethanol.

Helly's Modified Seawater Fixative

20 g Potassium dichromate
40 g Zinc chloride
800 ml Sterile seawater (natural filtered or synthetic)

Combine chemicals in a glass container and mix until dissolved. Use gloves and work safely with the zinc chloride. The solution can be stored until ready for use. Prior to using add:

12.5 ml 37 % Formalin

Combine and mix well. Add specimens and agitate gently every few hours if possible. Most small xeniid colonies are fixed in 12 to 20 hours. Rinse samples in running water for up to 8 hours or several changes of water over a 24-hour period. Place samples in ethanol. This may require a few changes also to remove any remaining fixative. Dispose of used fixative and wash containers thoroughly.

and less expensive. Remember that ethanol evaporates over time. Laboratory paraffin tape can be used to seal the lids to specimen jars, although it can be expensive or difficult to obtain at times. An inexpensive alternative is to place approximately five wraps of Teflon tape used for threaded plumbing fittings around the threads of the storage jar. This tape tends to break down when in contact with ethanol so the jar should be taped prior to adding the ethanol. After the ethanol and specimen are inside with a label place the lid on the jar. Then add about three to four wraps of electrical tape around the lid and in contact with the jar to create a complete seal. Small labels for inside the jar can be made from acid-free waterproof paper containing the specimen catalog number, date, identification, and any other pertinent information. Store the labels with the samples fully submerged in ethanol. An additional label placed on the outside of the storage container including specimen identification and a catalog number is also useful.

Sclerite Sampling

The skeletal elements of soft corals and gorgonians have an important place in their taxonomy. It is usually necessary to remove sclerites from the organic tissue matrix they are embedded in for examination. This is done to observe the fine detail located on the surface of sclerites and to measure them. There are two procedures that can be applied to sclerite removal. The first involves cutting away sections of tissue that include sclerites from either fresh or preserved samples and dissolving the tissue, allowing the sclerites to be observed individually and mounted. The second option is to mount a section of tissue on a slide and slowly dissolve the tissue to observe the arrangement and orientation of sclerites in their natural state.

The method outlined in here is used to extract large amounts of sclerites for individual examination, distribution to other institutions, or archiving for future reference.

Use caution when the hydrogen peroxide is added to the sclerites. The small amount of remaining sodium hypochlorite will exhibit a volatile reaction when the peroxide is added. Once the effervescence created by the reaction has subsided, gently swirl the vile to liberate any remaining dissolved gas then proceed with rinsing the sclerites. Whenever octocoral tissue is cut to remove sclerites, it is important not to make the cuts too shallow. A shallow sampling may cause larger surface sclerites to be broken or some smaller ones altogether missed. It is always better to cut surface samples a little deeper than too shallow. Figure 1 illustrates the sampling process. A second sample of the interior tissue will make it possible for comparing sclerites in surface tissue to those found deeper in the specimen.

Wet mounts are prepared by dissolving the sample directly on a microscope slide or well slide (Figure 2). There is often a significant

Sclerite isolation

20 ml 5.25 % Sodium hypochlorite (common bleach)
1 to 3 g sample tissue

Place sample in a test tube and add the sodium hypochlorite. Cover lightly with a small cloth or paper towel. This allows for gases to escape and prevents airborne dust from entering the tube. Let samples rest for up to 24 hours or until tissue is completely dissolved and bubbles stop forming at the surface. Agitate the test tubes by gently swirling ever few hours if possible. Siphon off sodium hypochlorite solution with a pipette. Leave sclerites that have settled to the bottom undisturbed. Then add:

0.5 ml Hydrogen peroxide (Buffered to a neutral pH)

Rinse sclerites by adding 20 ml of de-ionized or distilled water to the tube and stirring gently. Allow sclerites to settle out at the bottom. Siphon off the rinse water with a pipette. Repeat 5 to 7 times. Sclerites can be stored in small vials with 70 % ethanol.

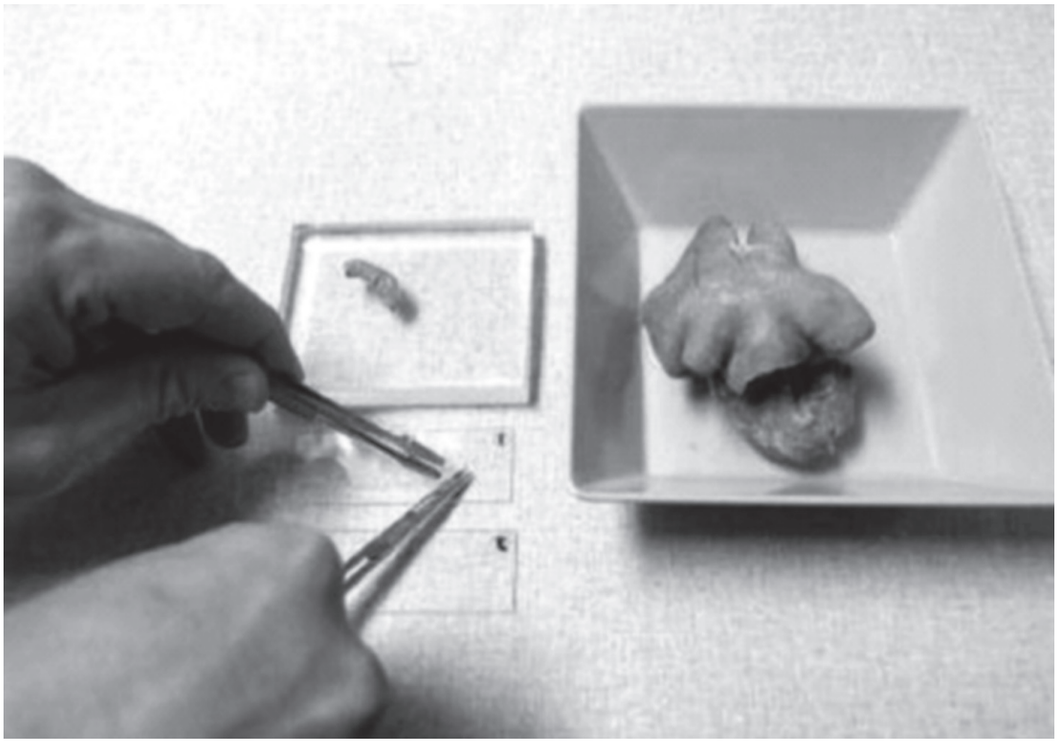

Figure 1. Sampling octocoral tissue.

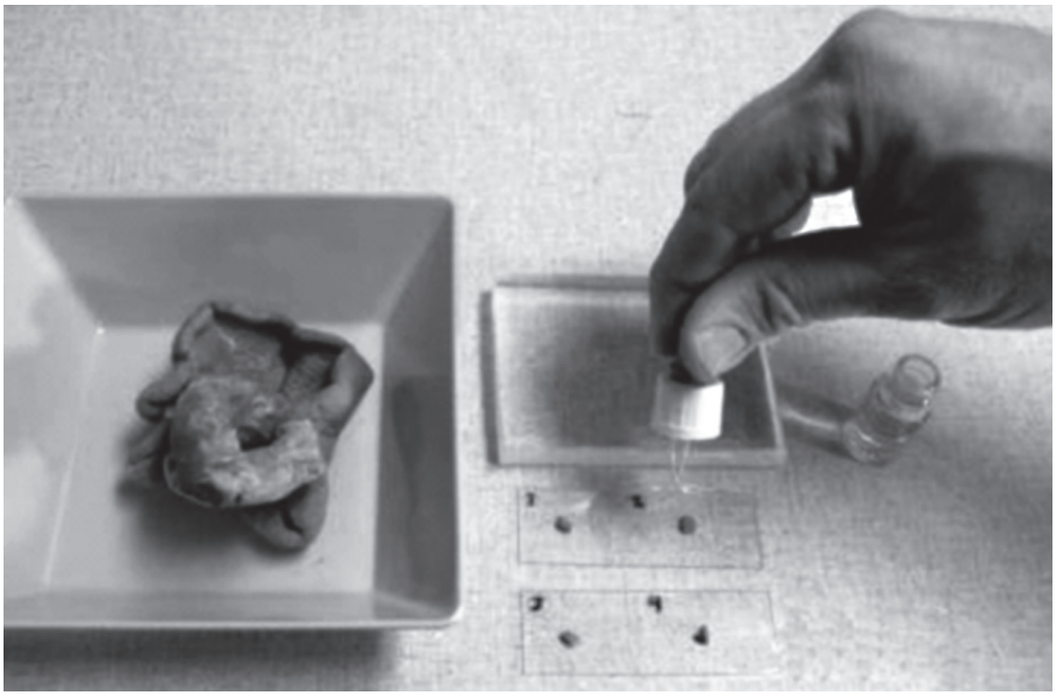

Figure 2. Removal of tissue through the addition of sodium hypochlorite.

amount of gasses released from the tissue of fleshy soft corals as it is dissolved. The bubbles shown in Figure 3 make it difficult to view the sclerites located underneath on the slide. A fine tipped teasing needle or dental tool works well to remove the bubbles. It is placed on top of the sodium hypochlorite droplet into the bubbles and then drawn off to one side (Figure 4). A small piece of tissue paper or paper towel laid against the bubbles can be used to break the surface tension and absorb the bubbles revealing the sclerites below. Isolated sclerites prepared as a wet mount may be oriented on the slide through the use of a cover slip. Place the slip over the

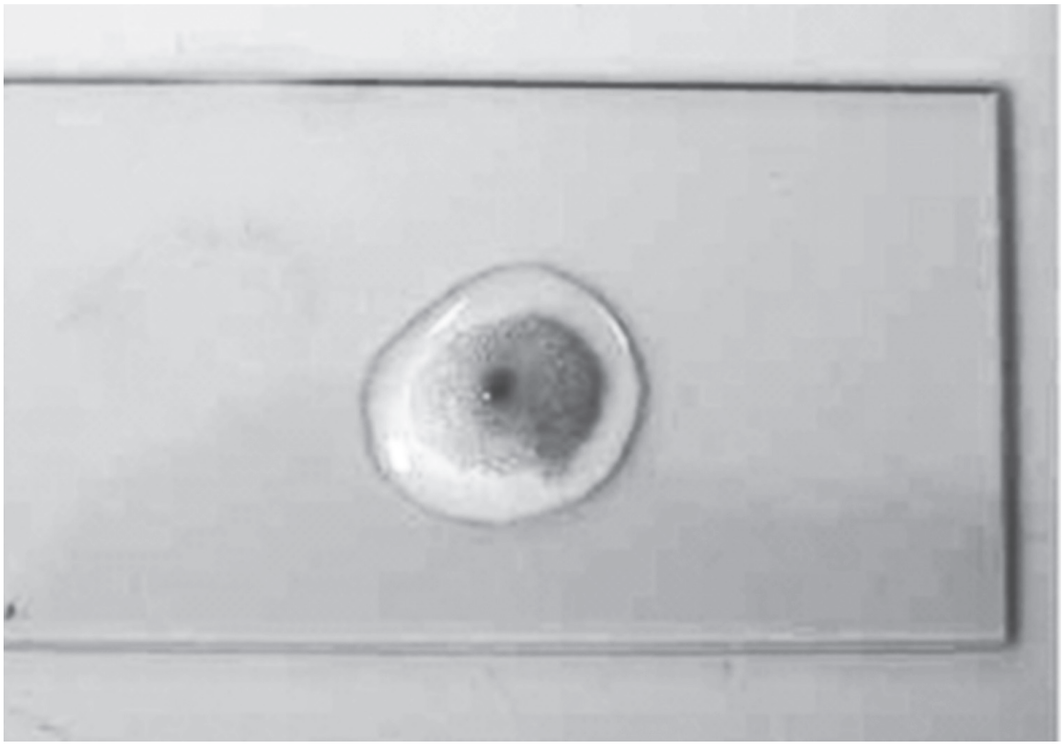

Figure 3. Gases formed from dissolved tissue.

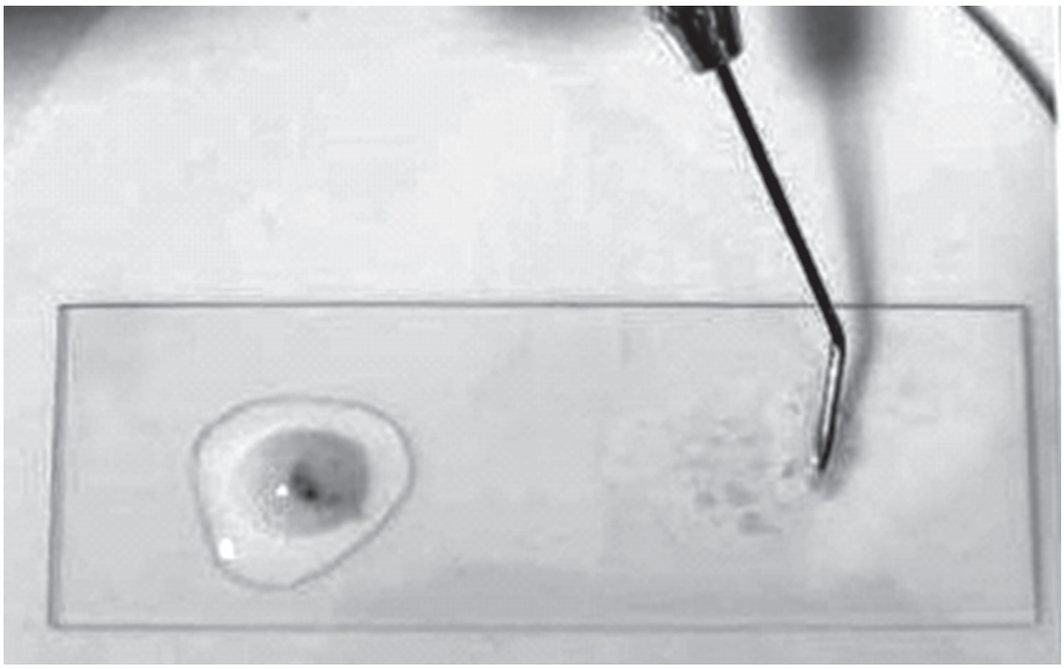

Figure 4. Bubble removal to reveal sclerites.

sclerites and use the tip of a needle to move the sclerites around on the slide for viewing.

A similar method to that described for sclerite isolation is used for examination of sclerite orientation and densities within the coenenchyme or polyp tissue.

The method clears the tissue over a period of minutes by using a diluted solvent. As the organic material disintegrates it reveals the position of sclerites and their abundance.

Although the diluted sodium hypochlorite or common bleach works well and is easily obtained it does rapidly disintegrate the tissue.

Sclerite orientation

5 ml 5.25% Sodium hypochlorite
15 ml De-ionized water

Prepare a diluted solution by combining sodium hypochlorite and de-ionized water in a vile. Place tissue sample on a well slide and add one drop of the prepared solution. Observe the slide under a compound microscope and continue to pipette the solution, drop wise, as needed. Allow a little time after each drop is added for the reaction to be completed. Once enough of the tissue has been dissolved to reveal the desired detail a cover slip can be added to the slide. Results can be recorded by photography but a drawing will capture better depth of field in a composite image.

Other clearing agents can be experimented with including potassium hydroxide, and phenol crystals dissolved in xylene. Always use extreme care when working with caustic chemicals and seek out laboratory assistance for advise on handling and disposal procedures.

Sclerite mounts

In most cases, a permanent record of the sclerites is needed. These can be very useful as a reference source, especially when comparing different specimens. Permanently mounted slides also transport well and are a good idea if the samples are to be shared with others or even deposited into a museum collection.

Selection of a mounting medium is an important step in preparing permanent slides. In order to view the mounted sclerites the media surrounding them has to have a different refractive index. Otherwise, it is difficult to resolve them under a binocular microscope. A medium with a significantly different refractive index than calcite will resolve the sclerites in the immediate field of view. The preparation given here is for the Durcupan mounting medium manufactured by Fluka. This is an Araldite based medium used in electron microscopy and comes in four parts. Only the first three parts are used for this application.

When Durcupan is used the drying times can be variable depending on the amount of humidity present. There is also a little variability in drying times from one batch to the next. Check the edge of a cover slip periodically and when the medium is hard to the touch, it is ready for storage. Durcupan components have a long shelf life, lasting for years, so it is best to prepare small quantities at a time. The process given in the text box below can be used for mounting mediums other than Durcupan. Experimentation will be needed to determine if the refractive index of other mediums is sufficient to provide enough contrast in the sclerite mounts.

Durcupan ACM Fluka medium preparation

10 ml Single component A/M Epoxy Resin
10 ml Single component B hardener
0.4 ml Single component C accelerator

Mark a small glass jar with 10 and 20 ml levels with de-ionized water. Dry the jar thoroughly. Add reagent “A” and “B” to the marks on the jar. Add reagent “C” and mix gently but completely avoiding any added air bubbles. Cover and store in the freezer at 0 °C. (32 °F.). Prior to use remove the medium from the freezer and warm to room temperature. This may take a few hours. Durcupan has a syrupy texture that becomes more viscous as it ages. Older mixed Durcupan may require warming over a flame for it to be usable. A new mixture should be made when it becomes too thick to work with. Store the mixture frozen after each use.

Slide preparation of sclerites

1. Pipette a few drops of sclerites stored in ethanol at the center of a clean microscope slide.
2. Place on a slide warmer or air dry until ethanol completely evaporates.
3. Add a drop of mounting media (Durcupan, etc…) to the top of the dried sclerites.
4. For large octocoral sclerites it may be necessary to mix them into the media with a warm needle. Try to make as few air bubbles as possible when mixing.
5. Place a square glass cover slip on top.
6. Label the microscope slide by hand or with a computer generated label using a small font. List all relevant information including scientific name, museum identification (if applicable), catalog number, and location within the specimen that the sclerites were taken from.
7. Dry microscope slides on a slide warmer or in an oven at 50-60 °C. for two to four hours or as needed. Cool to room temperature.
8. Store in a microscope slide case.
9. Duplicate slides of the same sclerite sample should be made if they are to be loaned out or deposited in a museum.

EXAMINATION

Gross examination

The primary features of a soft coral can be examined with little specialized equipment. A moderately powered stereomicroscope is useful in some cases. Preserved samples are removed from storage containers for study with little concern, provided they are kept free from contamination and kept moist. Dissection equipment, cutting blocks, and measuring equipment should all be cleaned between uses and between specimens. This becomes quite evident when microscopic observation of sclerites starts to reveal examples from a previous sample that were left on "contaminated" equipment. Samples placed under lamps may start to dry out as the ethanol evaporates. If this happens just place the sample back in its storage jar for a few minutes then remove it to continue working. Another option is to soak a piece of cheesecloth or similar material in ethanol and place it on the specimen in between periods of study. Common components of gross examination are measurements, colony shape, and photography.

Measuring a specimen is an important diagnostic tool for two reasons. First, it adds a sense of scale to larger sclerites visible to the unaided eye, which often form support bundles for polyps or ones that may fuse into a solid mass as in the case of some *Sinularia* species. Secondly, the size of a colony can assist in the identification process where some families or genera will contain unusually large or small species. All too often researches will post identification questions with photos in emails or over the Internet and it is difficult to determine sizes just from an image or brief description.

Recording colony shapes is not too difficult. Whether photographic or descriptive, the form of a colony should be as detailed as possible. Include the entire colony including basal attachments whenever possible. A poor example would be a photograph or measurement of a single gorgonian branch or a top view of a soft coral colony.

Digital imaging is a powerful means of recording and sharing octocoral details. Photographs of colony details such as autozooids, siphonozooids, calyces, and polyp arrangements can be made directly with a camera lens or attached to a Trinocular Stereomicroscope. A simple method for taking photographs through a standard stereomicroscope is afocal projection. Originally developed for astrophotography, this technique uses a digital camera mounted to a tripod that is placed directly up to the microscope ocular. The camera is adjusted to match the viewing angle of the ocular. Then by viewing through the camera's digital display images can be focused. Digital photography through a compound microscope is valuable for recording sclerite features. However, because there is a limit to the depth of field that can be recorded in an image multiple images may be necessary

to properly identify all the details in three dimensions. Modern software packages can be used to clean up digital images. Some, like Automontage ™ from the United Kingdom are capable of creating a composite image from multiple photographs. Multiple photographs can be used to develop a composite line drawing of sclerites that include the details obtained from photographs taken at different depths.

COMMON AQUARIUM OCTOCORALS

Modern public aquariums display a wide variety of zooxanthellate soft corals. With some basic laboratory equipment, a little time, reference materials and a genuine interest in soft corals professional aquarists can identify the corals they keep on display. Recent publications improve the accuracy of genus level identification (Fabricius and Alderslade, 2001; Janes and Wah, 2007). Also, general sclerite forms are given in table 1 for comparison of popular aquarium octocorals. Some of the more commonly exhibited tropical aquarium genera from the Pacific Ocean are presented here, highlighting their key attributes and differences. These comparisons are only given as a general guideline, as with most soft corals, there seems to be an exception somewhere or a species that just does not fit the common attributes of a genus.

Genus: *Lobophytum* (Marenzeller, 1886)
Large, fleshy colonies with primary unbranched

Table 1: Primary sclerite forms found in popular genera of soft coral maintained in aquaria.

	Sclerite shapes									
Genus	Strong Club	Weak Club	Spindle	Rod	Platelet	Capstan	Scaphoid	Double Head	Other	**Colored** *)
Soft Corals										
Lobophytum		X	X							
Sarcophyton	X		X							
Sinularia	X		X							
Cladiella					X			X		
Klyxum			X	X						
Nephthea			X			X				
Lemnalia				X						
Capnella	X					X				
Xenia					X					
Heteroxenia					X					
Anthelia				X						
Sansibia					X					
Clavularia			X	X						
Briareum			X							
Gorgonians										
Gorgonia			X				X			X
Pterogorgia			X	X			X			X
Pseudopterogorgia			X				X			X
Eunicea	X		X	X					X	X
Plexaura	X		X	X						X
Plexaurella			X						X	
Pseudoplexaura	X		X			X				X
Muricea			X			X				X

**) Column indicates some sclerites are colored but not necessarily all sclerites or all species in this genus.*

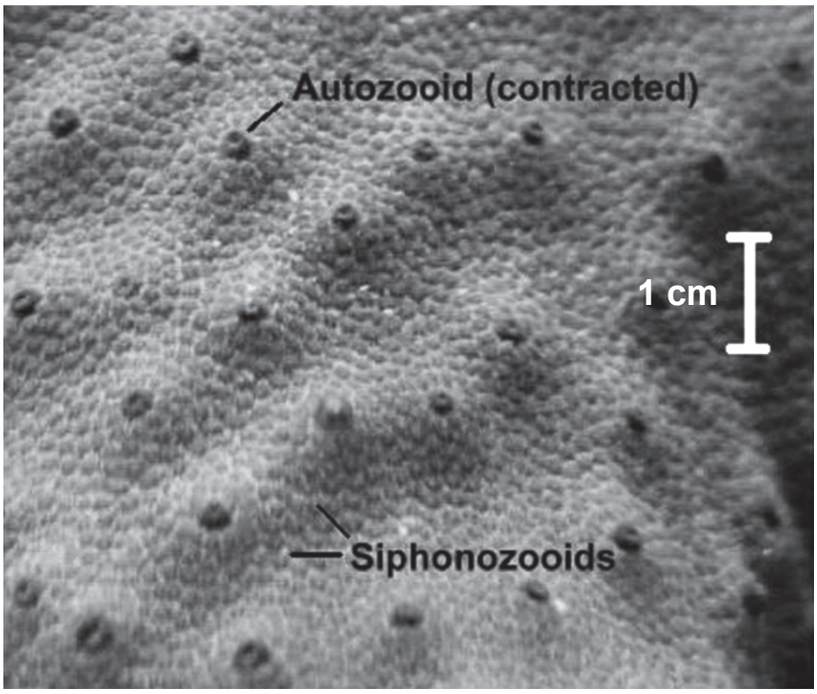

Figure 5. Typical distribution of autozooid and siphonozooid polyps in species of Sarcophyton and Lobophytum.

lobes are common ninety-nine percent of the time. Lobes may be absent in rare cases giving colonies a flat, smooth appearance. Lobes form from extended coenenchyme growing outward from the distal end of the stalk. Species of *Lobophytum* are firm to the touch and tend to grow encrusted on hard surfaces. Colonies contain very poorly formed clubs in the surface tissue. Interior sclerites are wide spindles with rows of well-defined warts or tubercles arranged in a girdle fashion around the sclerite. Differs from *Sarcophyton* in the detail of club sclerites, wider spindles and the lobed tissue. This genus differs prominently from *Sinularia* by the presence of siphonozooids (Figure 5), which are quite small and give the surface a fine dot-like appearance. Sclerites are colorless. Colonies are dimorphic.

Genus: *Sarcophyton* (Lesson, 1834)
Large, fleshy colonies with a mushroom shaped capitulum or polypary surface. The capitulum may be convoluted but is not lobed. The capitulum expands from a stalk that is free of polyps. Colonies contain slightly elongated, poorly formed club sclerites in the surface tissue. Narrow spindles in the interior have surface points or irregular warts on the surface, no girdles of warts. Sclerites are colorless. Most colonies produce a sheet of firm mucous at regular intervals. The mucous may impede attempts to coax out polyps when anaesthetizing specimens for fixation. Colonies are dimorphic with a similar surface appearance to *Lobophytum*. It is important to note that a recent molecular study recognized a possible third genus (Mcfadden *et al*., 2006). This third group included a mix of nominal *Sarcophyton* and *Lobophytum* species with intermediate morphologies. Many of the species in this mixed clade had a polypary that was difficult to distinguish from the stalk. Also, the surface sclerites were clubs with well defined heads.

Genus: *Sinularia* (May, 1898)
Many diverse growth forms with some exhibiting branched lobes. There are close to 130 nominal species of *Sinularia*. It differs from *Lobophytum* in the formation of branched lobes and it produces well-formed club sclerites. It is unlike *Sarcophyton* in colony morphology and the surface texture of the interior sclerites, which are complex, warty spindles. Sclerites are colorless. Colonies are monomorphic making them distinctly different from *Sarcophyton* and *Lobophytum* species when examined closely. Often the surface of the basal attachment will appear and feel rough. This is due to large sclerites, many of which will fuse together giving the base a cemented appearance. The fossilized form of the fused material is named Spiculite.

Genus: *Cladiella* (Gray, 1869)
Monomorphic polyps located in lobate, encrusting or stalked colonies. The polyps contain very small platelet sclerites that can sometime be difficult to locate. Sample a portion of surface tissue with some polyps to locate the polyp sclerites. Surface and interior sclerites nearly identical with surface sclerites being smaller. Polyps contain the majority of symbiotic algae so when they are retracted colonies appear whitish in color. The sclerites are colorless. *Cladiella* species are always soft and smooth to the touch because of their small sclerites.

Genus: *Klyxum* (Alderslade, 2000)
Soft, fleshy colonies with long, lobate branches covered in polyps. Some species produce copious amounts of mucous. Species of this genus are monomorphic. Polyp sclerites are typically scale-like or flattened rods. Occasionally longer rods may be found in the polyps. Descriptions in older literature refer to species as *Alcyonium* (Linnaeus, 1758), however true species of *Alcyonium* do not occur in the tropical Indo-Pacific. This is a new, recently described genus. Literature in the aquarium trade has historically attributed the popular Colt Coral to the genus *Cladiella* (Wilkens, 1992) but newer publications are now using the appropriate genus.

Genus: *Nephthea* (Audouin, 1826)
Arborescent colonies with polyps only located on the secondary branches, the stalks are smooth and sometimes semi-transparent. The polyps are monomorphic, have a short gastric canal, and do not retract. Under a stereomicroscope, supporting bundles of pointed spindle sclerites can be seen on the lower side of the polyps. Sclerites are colorless. Colonies are usually shades of brown with some yellow, green, or purple colors in life.

Genus: *Lemnalia* (Gray, 1868)
Long, narrow, colorless sclerites are found in the interior of *Lemnalia* colonies. Small monomorphic polyps line the secondary and fine branches. Zooxanthellate colonies are light brown to greenish in life and have a very strong odor. The long, smooth stalks and primary branches have a rough feel to the touch, similar to sand paper. Species of this genus differ distinctly from *Nephthea* and *Litophyton* when the sclerites are examined.

Genus: *Paralemnalia* (Kükenthal, 1913)
Digitiform branches rise from a common base. Retractile polyps line the branches giving them an asparagus-like appearance. Interior stalk sclerites are narrow with an assortment of fine, pointed protrusions on the surface. All the sclerites are colorless. Colonies are monomorphic. Although there is some diversity in colony forms, a more confusing aspect of this genus is species with retractile and non-retractile polyps. Further work is needed to sort out some species that may actually belong to the genus *Lemnalia*.

Genus: *Capnella* (Gray, 1869)
Colonies are lobed with polyps limited to the upper portion of lobes or branches. Monomorphic polyps are non-retractile so when they are disturbed, the tentacles fold over the mouth and polyps bend upward resting against the body wall. Sclerite shapes are rather distinct and very similar among species. One species, *C. imbricata* has leafy club sclerites in the polyps. Live colonies take on a grey color when the brown polyps contract.

Genus: *Clavularia* (Blainville, 1830)
Colonies are formed by shared, thin basal membranes known as stolons. Polyps retract into generally large calyces acting as a sheath to protect the polyps. Detritus, epiphytic algae and sometimes sponge often cover calyces and stolons. The calyx is usually rigid from long spindle sclerites in the tissue. Sclerites are colorless. The genus Hicksoni appears in older literature and was described based on the position of stolons relative to calyces. It is no longer valid. The presence of a calyx distinguishes *Clavularia* species from *Anthelia, Sansibia*, and other similar genera.

Genus: *Briareum* (Blainville, 1830)
This genus is transitional from soft coral to gorgonian with species displaying attributes of both groups. The encrusting basal membrane is made up of two layers, a cortex or surface layer and the medulla located underneath. Horizontal boundary canals divide the two layers. Calyces rise from the surface of the cortex and contain retractile polyps. Sclerites are magenta colored in the medulla and white or magenta in the cortex.
Colored sclerites are variable in their density and distribution within a colony. The types of spindle sclerites found in this genus have

unique shapes and surface architecture. Species from the genus *Solenopodium* and *Pachyclavularia* now belong to the genus *Briareum*. These two genera were originally based on gross morphological variations of *Briareum* and are no longer valid (Alderslade, pers. com.).

DISCUSSION

Both public aquariums and private individuals have successfully kept soft corals for many years. While some octocorals are regularly and correctly identified to the genus level, many are not. Modern aquarists realize that relying on only photographs to identify octocorals is often unsuccessful. For example, morphological features of *Sarcophyton* and *Lobophytum* can be too similar to make a clear distinction without a more thorough examination of the surface sclerites. In other cases, many aquarists have not adopted revisions such as the elimination of *Pachyclavularia* or the division of *Anthelia* and *Sansibia*. Perhaps this is due to limited access to updated literature and lack of a thorough understanding of the identification process needed to make accurate identifications.

Public aquarium personnel have the ability to contribute valuable data that might be shared through on-line databases and Internet resources. Recently acquired soft corals can be sampled for sclerites or in the case where multiple specimens are available; one of them should be fixed for analysis. Specimens that have been acquired from the wild as part of a collection expedition often yield important information on distribution locations. This could be compared with published biogeographical data when it exists or even contribute to new records of an octocoral family or genus in a particular area.

The techniques outlined in this paper provide a set of tools for the identification of octocoral genera. The materials needed to analyze soft corals and gorgonians are common in most public aquariums. Aquarium staff can apply the methods described here in a relatively short period of time to accurately identify octocorals. Genus level identifications are valuable when communicating the contents of a display, exchanging specimens with other institutions, or in the development of an experimental program. It is important to point out that working with genera is less of a time consuming task than the taxonomy of octocoral species. Working in the realm of Indo-Pacific octocoral species identification requires a greater commitment of time, resources, and access to older published literature than is commonly available.

At present, there is notable interest and funding available to conduct molecular research on corals. It is likely that the future of octocoral taxonomy will include advanced technologies like DNA sequencing. However, molecular biologists cannot distinguish and describe soft coral species solely on their molecular markers, traditional techniques are also required. There are no reliable markers yet available for the study of octocorals so a significant amount of research has yet to be done before molecular work has a prominent role in octocoral taxonomy. In addition to molecular work, another area of research for the next generation of investigators is intraspecific variations. It will be important for defining species in the future to look at what morphological ranges or limits exist. It is likely that some octocoral species described in the classical literature are actually variations of a single species.

Currently, there are significant challenges facing octocoral taxonomy. Many of the original published descriptions of soft coral species and even some genera are in doubt. There is a strong urgency to reevaluate museum specimens and the systematics of Indo-Pacific octocorals. However, limited funding, interest, and access to collections and older published literature make it difficult to do the work that is so desperately needed. In the not too distant future, the remaining few octocoral taxonomists working on species in the Indian Ocean, Red Sea, Malay Archipelago, and Pacific Ocean will be retired (Van Ofwegen, pers. com.). For now, there appear to be very few students of octocoral taxonomy that are accepting the challenge to find a career in soft coral systematics and still be able to provide for themselves and their families. The revisions needed to update the classification of octocoral species requires many hours of difficult work and access to museum collections spread around the world. The combination of limited funding, global distances between taxonomic collections, and a decline in the number of professional octocoral taxonomists suggests some creative solutions should be explored.

One short-term possibility is to increase our knowledge base of octocorals through communication among aquarists. This would expose more people to the process of octocoral taxonomy. Public aquariums offer a venue for

fostering new interest in the identification of octocorals. Staff members can be encouraged to collect samples and learn the techniques of identification. Communication is key. By sharing their findings, collections, observations, and questions it may be possible for aquarists to inspire a new generation of octocoral taxonomists.

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PERSONAL COMMUNICATIONS

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